MRID No. 427122-04

DATA EVALUATION RECORD

1. **CHEMICAL:** 2,4-D DEA.
Shaughnessey No. 030016.

2. **TEST MATERIAL:** Diethanolamine salt of 2,4-D; 73.8% active ingredient as salt; a light amber liquid.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: Duckweed (*Lemna gibba*).

4. **CITATION:** Thompson, S.G. and J.P. Swigert. 1993. Diethanolamine Salt of 2,4-D: A 14-Day Toxicity Test with Duckweed (*Lemna gibba* G3). Laboratory Project No. 281A-116. Conducted by Wildlife International Ltd., Easton, MD. Submitted by PBI/Gordon Corporation, Kansas City, MO. EPA MRID No. 427122-04. DP Barcode: D189984.

5. **REVIEWED BY:**

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Signature:

Date:

6. **APPROVED BY:**

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Senior Scientist
KBN Engineering and
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William Evans, Biologist
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Signature: William Evans

Date: 12/04/01

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study using a derivative substance (2,4-D DEA salt). Based on mean measured concentrations, the 14-day NOEC, LOEC, and EC_{50} for *L. gibba* exposed to 2,4-D DEA salt were 0.07, 0.13, and 0.44 mg ai/l, respectively.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

2-EEB-27 (1)

Reviewer: Dan Rieder

5/21/93
MRID No. 427122-04
AP-LEM

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5. **REVIEWED BY:**

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Supervisor, EEB/EFED
USEPA

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Date: 12/05/01

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study using a derivative substance (2,4-D DEA salt). Based on mean measured concentrations, the 14-day NOEC, LOEC, and EC_{50} for *L. gibba* exposed to 2,4-D DEA salt were 0.07, 0.13, and 0.44 mg ai/l, respectively.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

D
L

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** *Lemna gibba* G3 used in the test came from laboratory stock cultures. Cultures that had been actively growing for at least two weeks were used as test inoculum.

B. **Test System:** Test vessels used were 250-ml glass beakers. The test medium was M-Hoagland's medium (without EDTA or sucrose) with the pH adjusted to 5.0. The medium was autoclaved before use.

One-hundred milliliters of the appropriate test or control solution were placed into each beaker. The test vessels were kept at 25 ±2°C in a constant temperature room. The vessels were continuously illuminated at an intensity of 5.4-6.2 klux.

C. **Dosage:** Fourteen-day growth and reproduction test. Based on the results of a preliminary test, six nominal concentrations of 0.094, 0.19, 0.38, 0.75, 1.5, and 3.0 mg active ingredient (ai)/l were selected for the test. A medium control was also prepared.

A primary stock solution was prepared by dissolving the test material in medium. Five secondary stocks were produced in medium by two-fold serial dilution of the primary stock. The test solutions were prepared by diluting an appropriate volume of the stock solutions (1 ml) with medium to the final volume of 1 l.

D. **Test Design:** An inoculum of *Lemna gibba* consisting of 15-16 fronds, representing at least five plants, was added to each beaker (3 beakers per treatment). The beakers were indiscriminately positioned in the room. Frond counts were made on test days 3, 6, 9, 13, and 14. Observations of colony formation, tissue chlorosis and necrosis, root destruction, and changes in color were also made at these times.

The pH values of the initial and terminal treatment and control solutions were determined and the temperature was measured in a flask of water near the test vessels twice a day.

Samples of the test solutions were collected from freshly prepared medium on day 0 and from old solutions at test termination. The samples were analyzed for the

test material using liquid chromatography. Additionally, the lowest concentration stock solution was analyzed.

E. **Statistics:** Percentage growth inhibition was computed from frond number data. The 14-day EC_{50} and associated 95% confidence interval were calculated using the moving average angle method on percentage inhibition of frond growth versus day-0 measured concentration data. Plant and frond number, as well as percentage of dead, necrotic, and chlorotic fronds (for a total of 6 measured parameters) were also statistically analyzed. The no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) were determined by evaluating the effects on these parameters.

12. **REPORTED RESULTS:** The day-0 measured concentrations ranged between 84 and 104% of nominal, and day-14 samples ranged between 37 and 63% of nominal. The day-0 measured concentrations of 2,4-D DEA salt were 0.0794, 0.195, 0.380, 0.747, 1.52, and 3.11 mg ai/l (Table 1, attached). Analysis of the stock solution indicated that the concentration was 105% of nominal.

Percentage inhibition of frond number increased with increasing toxicant concentration and was significantly reduced ($p < 0.05$) in comparison to the control at the five highest treatment concentrations (Table 4, attached). The reduction at the lowest concentration level was considered to be treatment related.

Inhibition of plant number also increased with increasing toxicant concentration (Table 5, attached). Plant number was significantly reduced in comparison to the control at the five highest treatment concentrations (except at the 0.747 mg ai/l level). The reductions at these levels were also considered to be treatment related.

By day 13, colony breakup and root destruction was observed at the two highest treatment levels. There was a statistically significant increase in chlorotic fronds at these levels as well.

The pH was 5.0 in all treatment solutions and the control at test initiation and ranged from 6.5 to 6.7 at test termination. The temperature ranged from 23.4 to 24.3°C.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:
The 14-day EC_{50} was determined to be 0.60 mg ai/l with a 95% confidence interval of 0.55-0.66 mg ai/l based on frond production inhibition. The NOEC and LOEC were determined to be <0.0794 and 0.0794 mg ai/l, respectively.

Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

The type of lighting was not specified. Warm-white illumination is recommended.

The light intensity during the test (5.4-6.2 klux) was higher than recommended (5 klux).

B. Statistical Analysis: The reviewer based the analyses on mean measured concentrations rather than initial measured concentrations. The mean measured concentrations were 0.07, 0.13, 0.33, 0.59, 1.08, and 2.50 mg ai/l.

The reviewer used EPA's Toxanal program to determine the EC_{50} and analysis of variance (coupled with Dunnett's test) to verify the NOEC and LOEC. A more conservative EC_{50} was obtained using moving average angle analysis. The 14-day EC_{50} and 95% confidence interval were 0.44 mg ai/l and 0.34-0.58 mg ai/l, respectively. Since the results of Dunnett's test indicated that the frond number was not significantly different between the control and the lowest treatment level, the NOEC and LOEC were 0.07 and 0.13 mg ai/l, respectively.

C. Discussion/Results: This study is scientifically sound and meets the guideline requirements for a Tier 2 toxicity study using non-target aquatic plants. Based on mean measured concentrations, the 14-day NOEC, LOEC, and EC_{50} for *L. gibba* exposed to 2,4-D DEA salt were 0.07, 0.13, and 0.44 mg ai/l, respectively.

D. **Adequacy of the Study:**

(1) **Classification:** Core for a derivative substance (2,4-D DEA salt).

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 5-12-93.

Table 1
Summary of Analytical Chemistry Data

Sponsor: PBI/Gordon Corporation
Test Substance: Diethanolamine Salt of 2,4-D
Test Organism: Duckweed (Lemna gibba G3)
Dilution Water: M-Hoagland's Medium Without EDTA or Sucrose

Nominal Concentration (mg a.i./L)	Sampling Time (Days)	Measured[1,2] Concentration (mg a.i./L)	Mean
Negative Control	0[3]	<0.050	
	14[4]	<0.050	
0.094	0	0.0794	0.07
	14	0.0524	
0.19	0	0.195	0.13
	14	0.0699	
0.38	0	0.380	0.33
	14	0.288	
0.75	0	0.747	0.59
	14	0.426	
1.5	0	1.52	1.08
	14	0.633	
3.0	0	3.11	2.50
	14	1.88	
93.8	0	98.8	

[1] The Limit of Quantitation (LOQ) was based on the lowest matrix fortification level (0.050 mg a.i./L) extracted and analyzed with the samples.

[2] Measured concentrations were converted from 2,4-D to equivalent concentrations of diethanolamine salt of 2,4-D. Values less than the limit of quantitation (0.050 mg a.i./L) were not corrected for recovery.

[3] Samples were collected from the single batch of test solution prepared at test initiation to provide solutions for each of the three replicates per treatment.

[4] Samples were composites of the solution remaining in each of the three individual replicates per treatment pooled by concentration.

Table 4
Day 14 Frond Numbers, Mean Frond Numbers, and Percent Inhibition Values

Sponsor: PBI/Gordon Corporation
Test Substance: Diethanolamine Salt of 2,4-D
Test Organism: Duckweed (Lemna gibba G3)
Dilution Water: M-Hoagland's Medium Without EDTA or Sucrose

Day 0 Measured Concentration (mg a.i./L)	Replicate	Day 14 Frond Number	Mean Frond Number	Percent Inhibition
Negative Control	A	454		
	B	492	484	--
	C	505		
0.0794	A	435		
	B	368	409	15.5
	C	425		
0.195	A	263		
	B	403	292[1]	39.7
	C	209		
0.380	A	302		
	B	272	267[1]	44.8
	C	228		
0.747	A	216		
	B	227	233[1]	51.9
	C	257		
1.52	A	178		
	B	186	179[1]	63.0
	C	173		
3.11	A	145		
	B	168	154[1]	68.2
	C	149		

[1] Statistically significant ($p<0.05$) compared to the negative control replicates.

Table 5
Mean Numbers of Plants and Fronds Per Replicate

Sponsor: PBI/Gordon Corporation
Test Substance: Diethanolamine Salt of 2,4-D
Test Organism: Duckweed (Lemna gibba G3)
Dilution Water: M-Hoagland's Medium Without EDTA or Sucrose

Day 0 Measured Concentration (mg a.i./L)	Day 0 Numbers of		Day 3 Numbers of		Day 6 Numbers of		Day 9 Numbers of		Day 13 Numbers of		Day 14[1] Numbers of	
	Plants	Fronds	Plants	Fronds	Plants	Fronds	Plants	Fronds	Plants	Fronds	Plants	Fronds
Negative Control	5	15	6	39	18	124	46	176	119[2]	476[2]	128	484
0.0794	5	15	5	38	18	114	53	151	133[2]	531[2]	110	409
0.195	5	15	6	34	14	93	29	127	88[2]	352[2]	84[3]	292[3]
0.380	5	15	6	36	12	93	33	128	82[2]	327[2]	81[3]	267[3]
0.747	5	15	6	38	14	97	32	119	77[2]	309[2]	97	233[3]
1.52	5	15	5	34	12	80	30	95	83	144	90[3]	179[3]
3.11	5	15	5	35	12	77	26	89	73	126	65[3]	154[3]

[1] The duckweed plants were removed from the test chambers as counted in order to provide a more accurate representation of plant and frond numbers.

[2] The number of plants and fronds were estimated due to the large amount of duckweed present in the test chambers.

[3] Statistically significant ($p<0.05$) compared to the negative control replicates.

lemna frond number

Summary Statistics and ANOVA

Transformation = None

Group (Concentration (mg ai/l*))	n	Mean	s.d.	cv%
1 = control	3	483.6667	26.5016	5.5
2 0.07	3	409.3333	36.1432	8.8
3* 0.13	3	291.6667	100.1266	34.3
4* 0.33	3	267.3333	37.2201	13.9
5* 0.59	3	233.3333	21.2211	9.1
6* 1.08	3	179.0000	6.5574	3.7
7* 2.50	3	154.0000	12.2882	8.0

NOEC = 0.07 mg ai/l *

LOEC = 0.13 mg ai/l *

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

* mean measured conc.

Minumum detectable difference for Dunnett's test = -92.592360
This difference corresponds to -19.14 percent of control

Between groups sum of squares = 258817.333333 with 6 degrees of freedom.

Error mean square = 2009.095238 with 14 degrees of freedom.

Bartlett's test p-value for equality of variances = .029

MOSSLER 2 4 D DEA SALT LEMNA GIBBA 5-12-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
2.5	100	68	68	0
1.08	100	63	63	0
.59	100	52	52	0
.33	100	45	45	0
.13	100	40	40	0
.07	100	16	16	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .4998344

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	.1463942	.4454873	.3439413	.5847263

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.0605875	1	9.625381E-02

SLOPE = .8229426

95 PERCENT CONFIDENCE LIMITS = .6203791 AND 1.025506

LC50 = .5068454

95 PERCENT CONFIDENCE LIMITS = .3786097 AND .6945645

LC10 = 1.450885E-02

95 PERCENT CONFIDENCE LIMITS = 4.591721E-03 AND 2.967432E-02

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Ecological Effects Branch One-Liner Data Entry Form

Chemical 2,4-D DEA salt **Shaughnessy No.** 030016 **Pesticide Use** Herbicide

PHYTOTOXICITY AQUATIC SPECIES	% AI	EC_{50} (95%CL)	HRS/ DAYS	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. Lemna gibba	73.8	0.44mg ai/l (0.34-0.58)	14 days	0.07mg ai/l	1993/1993	427122-04 Core for A derivative substance	WIL	MM
2.								
3.								
4.								
5.								

COMMENTS: _____